SCIENTIFIC ABSTRACT

In this amended protocol which is a continuation of the trial proposed in the IND, we now propose to use myeloabation to increase the total number of CD4+ cells producing this anti-HIV-1 genetic antisense RNA. We will enroll subjects who are to be treated at the City of Hope with high dose chemotherapy and autologous stern cell rescue for HIV associated NonHodgkin's lymphoma. These subjects will be on antiretroviral therapy and will have already had treatment with standard dose chemotherapy and GCSF. Subjects will be entered into this protocol only after the appropriate number of PBSC has been isolated. At that time the PBSC will be transduced, and the transduced cells containing the anti-HIV-1 antisense genes will be infused into the HIV-1 subject from which they were originally derived.

The end points of this study are: the safety of the procedure; the extent of engraftment and proliferation of this engineered cell population and the relationship between the number of antisense RNA containing CD4+ cells and the viral load and CD4+ cell count.

The anti-HIV activity of the antisense transgenes was established in U937 cell line derived from human promonocytes that expresses the CD4 antigen and can be infected with certain strains of HIV-1. Genes coding for 3 distinct anti-HIV antisense sequences directed against 2 functional regions, tar and tat/rev, of HIV-1 were introduced into this cell line. Anti HIV antisense RNA transcripts appeared to accumulate in the nucleus. When these cells were subjected to 2 successive challenges with HIV-1 (BaL strain), the surviving cells continued to divide and grow at approximately the same rate and retained their CD4+ phenotype. Essentially all of the surviving cells produced high levels of antisense RNA when assayed using in situ hybridization. When these cells were again challenged with HIV-1 (again BaL strain) no detectable HIV-1-specific p24 antigen was observed, and PCRamplified HIV-1 nucleic acid sequences amplified from the gag gene were not detected. Also, the cells containing these three genetic antisense genes did not support the replication of three strains of HIV-1 isolated from individual patients (Liu et al., J Virol 71: 4079-4085,1997). These data indicate that the presence of intracellular anti-HIV-1 antisense RNA profoundly inhibited HIV-1 replication. As a further demonstration that these anti HIV antisense RNAs specifically blocked HIV-1 RNA transcription, tat-activated expression of the gene coding for chloramphenicol transacetylase driven by the HIV-1 LTR promoter was shown to be specifically inhibited in HeLa cells expressing either of the tat-antisense sequences or the tar-antisense sequence singly.

These three antisense sequences have been embedded into separate cloned human U1 RNA genes. These three U1/HIV-1 antisense genes were then combined into a triple U1/HIV-1 antisense cassette and incorporated into a Moloney Murine Leukemia Virus (MMLV)-based transducing vector (HGTV43). This murine retrovirus-based vector has the unusual property that it transduces CD34-enriched human cell populations in 18 hours without added cytokines and in the absence of any stromal cell feeder layer as well as it does in the presence of stromal cells and in the presence of IL3, IL6 and SCF and over the course of several days of culturing.

These data suggested that if enough CD4+ cells expressed anti-HIV-1 genetic antisense RNA the HIV-1 levels might be reduced in infected subjects. Accordingly we initiated a clinical trial (Human Gene Transfer Protocol No. 9801-230 of which this protocol is an amendment) in which

we transduced and subsequently reinfused PBSC into HIV-infected subjects using our transducing vector HGTV43. This phase 1 trial tested the safety of this procedure and the fate of the engineered cells (i.e. did they engraft, did they differentiate into CD4+ cells and did the antisense RNA gene continue to function). Finally we tested whether multiple infusions of transduced cells led to a higher extent of engraftment when compared with a single infusion.

The subjects of this trial were HIV positive with CD4+ cells counts above 200 cells/mm^3 . All were on anti retroviral therapy at the trial's inception. In this trial the numbers of viable PBSC induced and infused after transduction were within generally accepted ranges for published data on transduction with retrovirus-based transducing vectors.

No serious adverse events accompanied the infusion of these engineered cells. There was no change in either the viral load or CD4 + cell levels attributable to these engineered cells for time periods up to 1 year after infusion.

We assayed for the presence of the anti- HIV-1 genetic antisense RNA in bone marrow-derived stem cells (CD34+) as well as in CD4+ and PBMC over time after infusion. The assay procedure included RT-PCR to amplify the antisense sequences from RNA followed by restriction enzyme digest of the amplified material to confirm the presence of the original antisense RNA.

To summarize these results, we demonstrated long term survival of at least 6 months (21 months in 2 subjects, to date) of antisense RNA in a low number of bone marrow stem cells as well as cells in the peripheral blood mononuclear cell (PBMC) fraction and the CD4+ fraction. Since this low number of transduced PBMC and CD4+ cells has remained approximately constant over a number of months these data support the conclusion that stable engraftment of some of the antisense RNA-producing PBSC has occurred. The same conclusion can be drawn from the observation that bone marrow samples from the 5 subjects. Finally there was no evidence that multiple infusions (in 2 of the 5 patients) led to increased levels of engraftment.